Manipulating the hypoxic tumour microenvironment to study therapy resistance

Valorisation Addendum

Societal relevance:

Cancer is one of the leading causes of death worldwide and rising as a consequence of our ageing population. Cancer also has the highest direct and indirect costs of any disease. While developments have been made improving the survival rate of less malignant breast cancer patients, there is still a need to find new treatment strategies to improve survival further especially in patients with metastatic disease. In this thesis, we start by reviewing the current evidence for the role of Notch in breast cancer. Notch is cell to cell communication system that plays a critical role in development, determining cell fate and the generation of new blood vessels. Notch has been shown to act as both an oncogene and a tumour suppressor depending on the cancer type and context. Notch plays a role in normal breast development, but there is also overwhelming evidence that it also plays a role in the development and progression of breast cancer. This is not always due to mutations, with Notch signalling found to be active and cross-talking with many other oncogenic signalling pathways. For example there is evidence that both ER and EGFR signalling (both targets in breast cancer) negatively regulate NOTCH signalling. When inhibited (tamoxifen or anti-EGFR/ HER2) they activate Notch providing a survival signal. The co-targeting of EGFR and ER with Notch would sensitise the tumour to these treatments.

Scientific relevance:

Hypoxia (low oxygen) is a common feature of tumours due to their high metabolic activity, proliferation, and suboptimal vasculature. Hypoxia is also linked to metastasis which is the ultimate cause of treatment failure in the majority of cancers. While there are sometimes efficient treatments for early disease, the metastatic burden often leads to eventual treatment failure. Hypoxia is also known to reduce the efficacy of radiotherapy due to the oxygen effect enhancing the DNA damage created by radiotherapy. This means that more DNA damage and ultimately cell death is produced in well-oxygenated regions than in hypoxic regions. The hypoxic fraction of tumours has also been shown to be more resistant to chemotherapeutics through multiple mechanisms. Hypoxia also affects many of the same pathways as Notch signalling. Both play major roles in the maintenance of (cancer) stem cells and affect tumour progression and response to therapy. Both Notch and hypoxia play a critical role in angiogenesis, and in cancer this can lead to the formation of aberrant vasculature further increasing the hypoxic fraction of tumours. Overall hypoxia is a negative predictive factor and is associated with poor patient outcome. Due to this, there have been several different strategies aimed at relieving tumour hypoxia to increase the efficacy of radiotherapy and chemotherapy. In addition to this, hypoxia has also been used to target drugs to the tumour to reduce side effects which often limit the dose of chemotherapeutics that can be used, reducing their efficacy. Despite many promising candidates no hypoxia modification or targeting strategies have reached the clinic. Part of the reason for these failures is a lack of knowledge and understanding into how the hypoxic population of cells behave and how they contribute to therapy resistance and

tumour recurrence as a population and at the single-cell level especially in the complex *in vivo* microenvironment.

In this thesis, we describe the generation and development of a genetic tool to permanently label hypoxic cells in a temporally controlled manner. Using this tool we have shown that labelled cells are able to be visualised both in living tumours using intravital microscopy and once the tumour has been removed at single-cell resolution. We find that post-hypoxic cells were more proliferative than unlabelled cells raising questions as to how and what effect this may have on the tumour and patient outcome. The hypoxic tracer method provides a useful quantitative tool with high specificity and sensitivity to study hypoxic and post-hypoxic cell behaviour *in vivo* at the single-cell level. More information on the effect of hypoxia will lead to new and improved strategies for targeting these cells, which can eventually translate to the clinic. This tool will facilitate study into the dynamics of hypoxic and post-hypoxic cells within the primary tumour and their contribution to metastases. The effects of treatments such as radio and chemotherapy on this population of cells and how they respond can also be investigated.

We further developed the tool to allow the selective killing of labelled cells allowing investigation into the role of hypoxic cells in therapy resistance. This system will be able to show what effects targeting the hypoxic population will have, and what benefits this has in combination with established treatments. This tool will also be useful in understanding the efficacy of hypoxia modification and targeting strategies. It will facilitate visualisation of the hypoxic population with and without these strategies and what effect they have on this population of cells. It will help in the optimisation of these strategies and how and when they are applied. The timing of these strategies in relation to other treatments such as radiotherapy can be investigated to elucidate the most effective schedule.

While cell lines are an indispensable tool in the study of cancer they often do not allow the use of immunocompetent mice or effectively replicate the clinical situation even in orthotopic models. To overcome this limitation we created a knockin transgenic mouse strain with this tool to facilitate the study of hypoxia in spontaneous tumours when crossed with mice lines such as MMTV-PYMT; a transgenic mouse model which spontaneously develop ductal mammary carcinomas. Spontaneous tumours better imitate the clinical situation due to undergoing initiation steps such as immunoediting as well as being influenced by organ-specific microenvironmental factors such as hormones and tissue architecture. This model can therefore give data that is more relevant to the clinical situation. In addition to applications in cancer research, this tool will be of use in other areas where hypoxia is a factor. Other problems such as tissue ischemia (heart failure, stroke) lead to hypoxia, where this tool can be of use to study the affected cells and tissues. On top of this certain aspects of development are dependent on hypoxia and hypoxia-inducible factors, with human embryos developing in a predominantly hypoxic environment.

Together, the work in this thesis provides a rationale for the use of Notch inhibitors in the treatment of breast cancers and provides the scientific community with a valuable new tool.